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Acute Toxicity and Ethological Responses of *Channa punctatus* (Bloch) Exposed to an Organophosphorus Insecticide, Dimethoate

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Abstract: Dimethoate is a commonly used pesticide in the agricultural land. The present investigation was undertaken to evaluate the acute toxicity of dimethoate, an organophosphorus insecticide, in freshwater fish *Channa punctatus* (Bloch) and to study a relative behavioural changes after exposing for 24, 48, 72 and 96 h. The regression equation calculated for 24, 48, 72 and 96 h was found as Y= -0.50+9.05X, Y= -0.12+8.21X, Y= 0.27+7.45X and Y= 0.68+7.64X, respectively whereas the LC_{50} value calculated after 24, 48, 72 and 96 h was at 1.136, 1.034, 0.921 and 0.817 ml/L, respectively. During the static bioassay test, water quality parameters showed slight fluctuation indicating that the mortality of test fishes occurred due to pesticide exposure and not due to alterations in water quality. During dimethoate exposure, fish showed various ethological responses such as faster opercular movements, convulsions, excessive mucus secretion and loss of equilibrium or imbalanced swimming. Prior to death, the clinical signs like pale body colour and hemorrhagic patches over the body were noticed which become progressively more prominent up to the end of the experiment. Result of this investigation showed that dimethoate pesticide is potentially toxic to *Channa punctatus* so, the natural waterbodies must be monitored at regular interval to reduce the impact across food chain.

Keywords: Acute toxicity, Ethological responses, *Channa punctatus*, Dimethoate

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Introduction

Pesticides, which are biological toxicants, are utilized by humans for preventing, destroying, repelling, mitigating or eliminating any pest (insect, termites, mites, nematodes, weeds, herbs, fungus, rats etc.) and to increase crop yields, as well as to control the spread of disease by insect vectors. It includes insecticides, nematicides, weedicides, herbicides, fungicides, rodenticides, plant growth regulators and various other substances to control pests (Gervas *et al.*, 2021; Naz *et al.*, 2023). Pesticides are also classified on the basis of their composition named:

Organophosphates, pyrethroids, organochlorines and carbamates. Some pesticides are soluble in water while others are soluble in organic solvents and perform various functions on the basis of their composition (Dad *et al*., 2022). The use of pesticides has resulted in significant environmental and health risks for various organisms, including humans.

Hoegberg and Cassaday (1951) were the first to describe dimethoate, an organophosphate insecticide, which was first made available on the market in 1965 under the brand name Rogor (Bhandare *et al*., 2011). Dimethoate inhibit the enzyme acetyl cholinesterase, which accumulates in the synapses of the central and peripheral nervous system. Consequently, there are indications of cholinergic neurotoxicity and an over activation of postsynaptic cholinergic receptors (Srivastava *et al.*, 2010). Due to its high solubility in water, dimethoate can leach into nearby waterbodies and affect aquatic life including fishes.

In agricultural land the use of pesticide has become a common practice to increase the crop yield through controlling pest and insect vectors to control the spread of pest related diseases (Prakash and Verma, 2014). The contamination by pesticides from agricultural fields is a serious problem of water pollution and its long-term effect may result in the loss of aquatic biodiversity (Jothi and Narayan, 1999). Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only threatened their lives but also eventually enter the food chain leading to serious public health hazards (Ilavazhahan *et al.*, 2010). Unfortunately, the indiscriminate use of these pesticides to improve agricultural production and yield may have impacts on non-target organism, especially aquatic life and environment (Nwani *et al.* 2010 a,b).

The enormous use of pesticides becomes the predominant environmental contaminants and brought adverse influence on agro-ecosystems. It also caused alteration in physiological processes of non-target organisms (Verma and Prakash, 2018). Once these pesticides are released in the environments they are metabolized in short time whereas other persists over longer period and can accumulate in the soil and water and badly influence the widespread biodiversity and its buffering mechanism (Prakash and Verma, 2020).

In addition to causing death, fish behaviours are altered by changes in the physio-chemical and biological characteristics of the water. Fish are more vulnerable to contaminants than other aquatic life, and modifications in behaviours in fish have been thought to be a sensitive sign of toxicity (Yadav *et al.*, 2007). An organism may adapt to both internal and external stimuli through ethological responses, which helps it overcome the difficulties of existing in a changing environment. Because these fish behavioural anomalies might give appropriate indications regarding the environmental circumstances, so can be used as biomarkers (Dhanalakshmi *et al*., 2018).

Toxicity tests are experiments designed to predict the concentrations of toxicant and its duration of exposure required to produce an effect. Toxicity is species-specific because individuals have different levels of response to the same dose of a toxic substance (Smith and Stratton, 1986). The toxicity bioassays are used to detect and to calculate the potential toxicological effects of chemicals on organisms. These tests provide a data base that can be used to assess the risk associated with a situation in which the organisms live. A variety of methods have been developed to evaluate the hazard and potential toxicity of chemicals to organisms, such as acute toxicity test, sub-acute toxicity test or chronic toxicity test. LC_{50} is the estimation of the dose or concentration necessary to kill 50% of a large population of the test species. Experimentally, this is done by administrating a toxicant at different concentration to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48, 72 and 96 h. The acute toxicity data are important and beneficial in the fixation of sublethal concentrations for chronic toxicity tests.

Fishes are very sensitive to a wide variety of toxicants such as pesticides in water (Heger *et al*., 1995). By altering the activities of numerous enzymes and metabolites, pesticide accumulation in tissues causes a variety of physiological and biochemical alterations in fish and other aquatic animals (Nagarathnamma and Ramamurthi 1982). Fish serves as a bioindicator species as it responds with great sensitivity to changes in the aquatic environment and thus, has an important role in the monitoring of water pollution (Srivastava *et al*., 2010).

Probit analysis is a type of regression used to analyse binomial response variables. Probit analysis is commonly used in toxicology to determine the relative toxicity of toxicant or pollutant to living organism (Singh and Zahra, 2017). This is done by testing the response of an animal at different concentrations of toxicant and then comparing the concentrations at which a response occurs. Probit method is widely accepted and most accurate method for calculating LC_{50}. The median tolerance limit of any pollutant is meant as an elementary guide in the field of ecotoxicology and without reference to the median tolerance limit, no information on sublethal effects can be deduced (Jammu *et al.,* 2023). Therefore, the present study is aimed to examine the acute toxicity (LC_{50}) of dimethoate, an organophosphorus pesticide on fresh water snake headed fish, *Channa punctatus*, by using SPSS and also to study the ethological responses due to its toxic effects. The study will provide baseline data that might be utilized to determine the relative sensitivity of dimethoate pesticide and help further in determining the acceptable level dose or concentration.

Materials and Methods

The freshwater fish, *Channa punctatus* (Bloch) were collected from local fresh waterbodies in and around Sultanpur and the fish of uniform length (10.7±0.20 cm) and weight (15.5±1g) were selected for the bioassay experiment. Then these fishes were carefully brought to the laboratory in plastic container, avoiding any injury during transportation. The collected fishes were washed with 1.0 % solution of $KMnO_4$ for 5 min to remove any dermal infection. Fish were acclimated to laboratory conditions for two weeks at room temperature (26 ± 1.4°C) prior to experimentation. All the necessary precautions were taken to keep the aquaria clean and free from mechanical disturbances. All the fishes were regularly fed with commercially food pellets and medium (tap water) was renewed every two days to remove faeces and food remnants. Feeding was stopped 24 h prior to the toxicity test.

The various physico-chemical parameters such as temperature, pH, dissolved oxygen, hardness and total dissolved solids were recorded daily with the help of digital Water Quality Meter (Model: ISO-Tech System (ITS)-901) using glass electrode. Free carbon dioxide was measured by using phenolphthalein indicator and sodium hydroxide titrant.

The experiments were carried out in glass aquaria of 15 L capacity in laboratory conditions. The stock solution was prepared by dissolving dimethoate (30%EC, Rallis India Ltd. Mumbai-India with 85% purity) in distilled or deionized water. The range finding tests or exploratory tests was carried out to determine the final concentrations to be used in definitive test by following the method prescribed by APHA (1998). The fishes were exposed to 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75 and 3.0 ml/L concentration of dimethoate in the exploratory test. Each group was placed in a glass aquaria containing 10 L of water. At concentration 1.6 ml/L, 100% mortality was found within 24 h. and no mortality was found up to 96 h at concentrations below 0.60 ml/L. After determining the range, fish were exposed to final concentration of 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 1.10, 1.20, 1.30, 1.40 1.50 and 1.60 ml/L.

Fishes were exposed to each concentration in triplicate and the assays were carried out up to

Table 1: Percentage of mortality of fish, *Channa punctatus* (Bloch) at different concentrations of dimethoate (ml/L) at different exposure period

Concentration of Dimethoate (ml/L)	Log Conc. of Dimethoate	No. of Test Fish	Percent Mortality at different Exposure Period (h)			
			24	48	72	96
Control (0.0)	-	10	0	0	0	0
0.50	-0.301	10	0	0	0	0
0.60	-0.222	10	0	0	10	10
0.70	-0.155	10	0	10	20	20
0.80	-0.097	10	10	20	30	30
0.90	-0.046	10	20	30	40	40
1.00	0.000	10	30	40	60	60
1.10	0.041	10	40	50	70	70
1.20	0.079	10	50	70	80	90
1.30	0.114	10	70	80	90	100
1.40	0.146	10	80	90	100	-
1.50	0.176	10	90	100	-	-
1.60	0.204	10	100	-	-	-

96 h and dead fish were removed as the experiment proceeded. The fish were considered dead, if there was any sign of immobility, loss of equilibrium, lack of opercular movement, or illness or exhibited no response when probed with a glass rod. As dead fish deplete the dissolved oxygen, they were removed immediately. The number of dead fish per group was recorded after 24, 48, 72 and 96 h of exposure and their mortality computed in a tabular form, as specified by Sprague (1969). The per cent mortality observed for each concentration was calculated and converted to probits utilizing SPSS software version 26. The LC_{50} values (with 95% confidence limits), the correlation between mortality (Probit) against the log10 concentrations and the regression line equation were also obtained through the best-fit line. During the experiment, ethological responses of test fish, *Channa punctatus* exposed to sub-lethal concentrations of dimethoate were also critically observed. During the experimental period, no death was recorded in control set-up. Additionally, behavioral reactions such as increased mucus production, altered swimming pattern, loss of scale, and altered color patterns were taken into account and closely observed during the entire period of experiment.

Results

Median Lethal Concentration (LC_{50}) of dimethoate:

No mortality of *Channa punctatus* (Bloch) was observed at 0.7, 0.6, 0.5 and 0.5 ml/L after exposure to 24, 48, 72 and 96 h, respectively whereas 100% mortality were recorded at 1.60, 1.50, 1.40 and 1.30 ml/L after exposure to 24, 48, 72 and 96 h, respectively (Table 1). However, fishes exposed to dechlorinated tap-water were observed to be healthy and normal. The median lethal concentration (LC_{50}) values of dimethoate for 24, 48, 72 and 96 h and their respective 95% confidence limits, regression equation and coefficient of determination are given in Table 2 as calculated by and converted to probits utilizing SPSS software version 26. LC_{50} values at 24, 48, 72 and 96 h for *Channa punctatus* after acute exposure to dimethoate were 1.136, 1.034, 0.921 and 0.817 ml/L, respectively. A strong correlation between the log dose and probit mortality of the fishes was observed (Table 2). The plot of adjusted probits and predicted regression line for 24, 48, 72 and 96 h dimethoate toxicity to *Channa punctatus* are depicted in Figures 1-4, respectively.

Water quality of aquarium waters during dimethoate bioassay:

Table 2: LC_{50} values with 95% confidence limits for *Channa punctatus* (Bloch) exposed to dimethoate at different exposure period

Exposure Period (h)	LC_{50} Values of Dimethoate (ml/L)	95% Confidence limits for Concentration (ml/L)		Regression Equation	Chi Square Value (P value)	Coefficient of determination (R^2Linear)
		Lower Limit	Upper Limit			
24	1.136	1.057	1.219	Y= -0.50+9.05X	0.1.899 (0.997)	0.979
48	1.034	0.954	1.117	Y= -0.12+8.21X	1.961 (0.992)	0.976
72	0.921	0.840	1.003	Y= 0.27+7.45X	1.575 (0.991)	0.985
96	0.817	0.743	0.893	Y= 0.68+7.64X	2.560 (0.862)	0.940

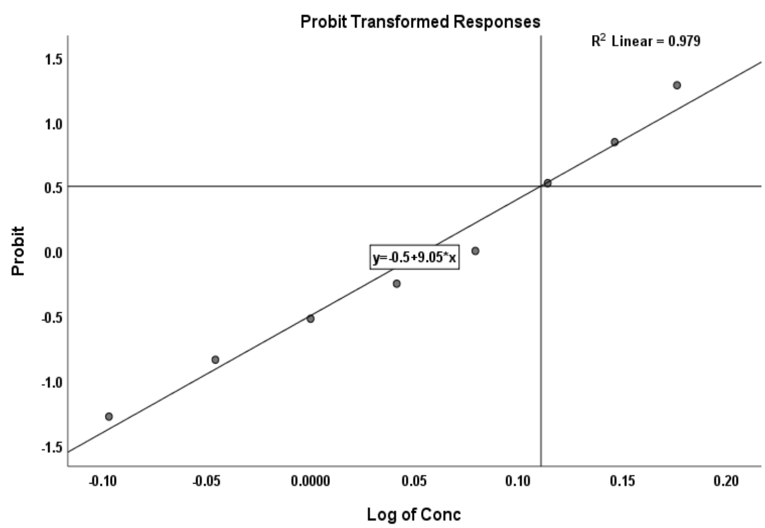

Fig. 1: Regression Line (based on Probit analysis) of Log concentration of dimethoate vs % mortality of *Channa punctata* (Bloch) (at 24 h).

The physico-chemical parameters assessed during static bioassay of dimethoate showed slight variation up to the completion of bioassay. The temperature, pH, DO, Free CO_2, hardness and TDS of water ranged from 27.2 to 27.4^{0}C, 7.5 to 7.8, 7.02 to 7.98 mg/L, 1.80 to 1.86 mg/L, 80.4 to 82.4 mg/L and 205.62 to 211.13 mg/L, respectively (Table3).The dissolved oxygen content decline slightly but it seems to be insignificant (Table 3).

Behavioural responses in fishes exposed to dimethoate:

The behavioural responses of the test fish, *Channa punctatus* exposed to pesticide was found to depend on its concentration and duration of exposure period. There were no behavioural alterations observed in the control group. The behavioural responses showed by *Channa punctatus* during acute exposure to dimethoate are presented in Table 4. Loss of balance was seen clearly during 12 h of experiment in the fishes which later on died. Those fishes which survived during entire bioassay test did not show the sign

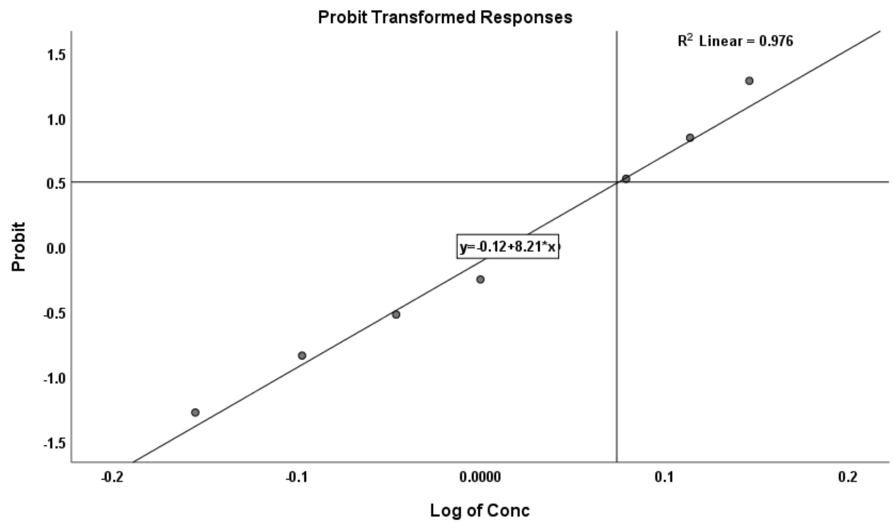

Fig. 2: Regression Line (based on Probit analysis) of Log concentration of dimethoate vs % mortality of *Channa punctata* (Bloch) (at 48 h).

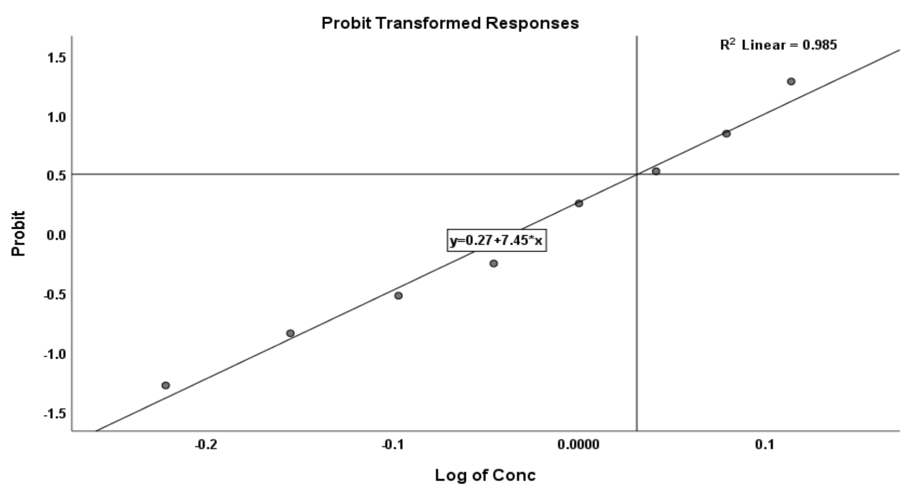

Fig. 3: Regression Line (based on Probit analysis) of Log concentration of dimethoate vs % mortality of *Channa punctata* (Bloch) (at 72 h).

of imbalance swimming. Loss of balance or imbalance swimming just started after 12 h of the commencement of the experiment which grew intense with erratic, irregular and jerky movements. Convulsions were seen to get started from 12 h, progressively increase up to 24 h and lasted until 48 h. The slight mucus secretion could be seen from 24 h that increases progressively up to the end of experiment. Initially opercular movements, gulping activity and fin movement were increased followed by decrease. Prior to death, fishes become inactive, nonmotile, body becomes pale in colour and haemorrhagic patches were seen on the ventral side of the body.

Discussion

Acute toxicity shows susceptibility of fish to particular toxicants and reflect their survival potential. Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the

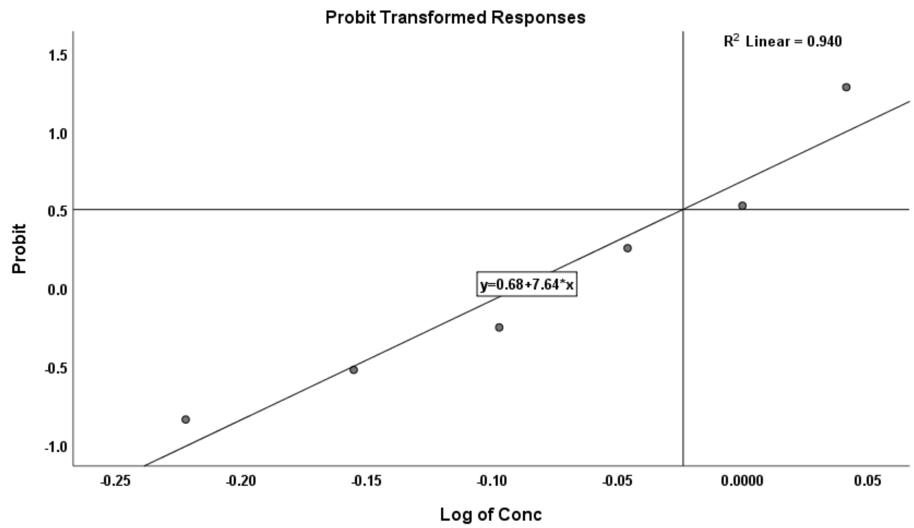

Fig. 4: Regression Line (based on Probit analysis) of Log concentration of dimethoate vs % mortality of *Channa punctata* (Bloch) (at 96 h).

Table 3: Water quality parameters during dimethoate bioassay on different intervals

S. No.	Physico-chemical parameters of Water	Exposure Period			
		24 h	48 h	72 h	96 h
1	Temperature (° C)	27.4±0.16	27.3±0.25	27.5±0.16	27.2±0.24
2	pH	7.8±0.08	7.6±0.16	7.7±0.12	7.5±0.08
3	Dissolved Oxygen(mg/L)	7.98±0.05	7.39±0.02	7.12±0.10	7.02±0.02
4	Free CO_2 (mg/L)	1.80±0.16	1.82±0.10	1.84±0.16	1.86±0.05
5	Hardness (mg/L)	82.40±0.49	80.87±0.25	81.4±0.25	80.4±0.16
6	Total dissolved solids(mg/L)	205.62±0.26	209.42±0.25	211.13±0.25	208.12±0.05

Table 4: Behavioural responses elicited by *Channa punctatus* during bioassay

Time	Opercular movements	Convulsions	Mucus Secretion	Loss of Balance	Surfacing &Gulping activity	Fin movements
00 h	-	-	-	-	-	-
12 h	++++	+	-	+	+	+
24 h	+++	++	+	++	++	++
48 h	++	+	++	+++	+++	+++
72 h	++	-	+++	+++	++	++
96 h	+	-	+++	+++	++	+

- Normal; + Feeble; ++ Progressive; +++ Intense; ++++ Severe

toxicant, its concentration and duration of exposure (Singh *et al.,* 2009). During the present investigation, static bioassay test was carried out in *Channa punctatus* (Bloch) exposed to dimethoate. The 96 h LC_{50} for dimethoate was found to be 0.817 ml/L, which is in accordance with the reports of earlier researchers. The 96 h LC_{50} value of 21.27 ppm (Paithane *et al.*, 2012) while 0.75 ml/L (Sarma *et al.*, 2013), 19.10 µg/L (Ali *et al.,* 2014), 47 mg/L (Dikshith and Raizada,1981) and 65 mg/L (Srivastava and Singh, 2001) has been reported for *Channa punctatus* exposed to dimethoate. However, 24 h LC_{50} value of 3 ppm (Shereena *et al.*, 2009) and 17.92 mg/l

(Srivastava and Singh, 2001) has been reported for *Tilapia mossambica* and *Channa punctatus,* respectively exposed to dimethoate indicating that the test fish is much susceptible to the dimethoate toxicity. The low values of LC_{50} found during present investigation are attributed to medium size of fishes (10.7±0.20 cm length and 15.5±1 g weight) which have potentially weak immune system to carry out the elimination of the toxicant from the body. Furthermore, because a toxicant's absorption is closely correlated with fish size, the quick distribution of dimethoate pesticide in small sized fish causes behavioral abnormalities to occur faster than the normal (Qayoom *et al.,* 2016a). Depending on the size, age, and condition of the test species as well as other experimental variables, LC_{50} values also varying, even for a single species and same toxicant (Sprague, 1969). The results of present investigation revealed that the mortality rate increased with increasing concentration of dimethoate and for a particular concentration the mortality rate increase with increasing period of exposure, depicting a direct correlation betweenthe mortality and the concentration.Similar observations were found by some researchers (Yadav *et al*, 2007; Pandey *et al*., 2009; Tiwari and Prakash, 2021). This reflects the regular mode of action, due to pesticide that cause death of fish. The death of fish could be due to the lethal action of pesticide that causes alterations in physiological and biochemical process related to cellular metabolic pathway. The toxicity of any toxicant for fish depends on species, sex, age, weight, dose or concentration, exposure period, organic or inorganic form etc. (Amsath *et al*., 2017; Tiwari and Prakash, 2021).

The physico-chemical parameters of aquarium water showed slight alterations during the entire period of acute toxicity test because of normal atmospheric temperature and nonavailability of feed to fishes during the experiments. Moreover, feeding stopped one day prior to commencement of the experiment which prevented the deterioration of water quality (Qayoom *et al.*, 2016b). Thus, it can be suggested that the hypoxic conditions did not prevail in the aquarium water during acute toxicity test and mortality of test fish was merely due to the dimethoate intoxication. Another reason of fish mortality might be excessive secretion of mucous which decreases the gaseous exchange through gills that reduces the ability of fish to utilize dissolved oxygen from the water. The values of dissolved oxygen remained more or less constant throughout the experiment signifying nonhypoxic conditions in the aquarium throughout the assay. *Channa punctatus* being an air breathing fish it takes atmospheric oxygen i.e. aerial respiration, therefore the changes in dissolved oxygen appears inconspicuous. The increase in TDS of aquarium water on 2^{nd} and 3^{rd} days could be attributed to the fact that the feed left in the intestine of fish might have passed out in the water resulting to increase the TDS of aquarium water. The increase in TDS could also be due to the pesticide concentration in the aquarium.

The changes in the behavioural responses are the most sensitive indication of potential toxic effects of any pollutant. In the present study, the behavioural changes noticed in experimental fish increased with increasing the concentration of dimethoate pesticide and duration of exposure (24 to 96 h). Any behavioural change in fish indicates deterioration of their habitat due to the effect of dimethoate pesticide (Sharma *et al*., 2022). The fish exposed to dimethoate showed abnormal behavioral responses in the form of hyperactivity (erratic swimming with surface activity for gulping air), enhanced convulsion, loss of equilibrium, reduced fin movements. In the present study, the convulsion in the initial stage of acute toxicity test may be attributed to the accumulation of acetylcholine in nerve synapses because of inhibitory nature of organophosphate pesticide to acetylcholinesterase (AChE) enzyme in the synaptic cleft which leads to paralysis in bioassay exposure (Qayoom *et al*., 2016a). Upon exposure to the pesticide, increase in surfacing for gulping of surface water appears to be an attempt by the fish to avoid breathing in the contaminated or polluted water. Similar observation has been reported in freshwater fishes after exposure to dimethoate, an organophosphate pesticide (Singh *et al*., 2009; Pandey *et al*., 2009). In the present investigation, initially the operculum movement increased suddenly but progressively it was decreased with exposure time. Initial increase might be due to sudden response to shock or stress. Decreased opercular movement helps the fish to improve resistance against toxic stress caused by the dimethoate by reducing the absorption of toxicant through the gill and to increase the survival period of fish in toxic environment (Singh *et al*., 2009; Pandey *et al*., 2009; Singh and Kumar, 2013). Excess mucous secretion over the body after dimethoate exposure is the first line defence mechanism reactions elicited by the fish towards the stress conditions or to counter the effect of pesticide on body surface and mucous membrane (Üner *et al*., 2006; Singh *et al*., 2009; Pandey *et al*., 2009; Srivastava *et al*., 2010; Qayoom *et al.*, 2016a). The loss of equilibrium status or balance in pesticide exposed fishes noticed in the present study may be due to nonfunctioning of the brain or due to variation in enzymatic activities and biogenic amine (Yadav *et al*., 2005, 2007; Tiwari and Prakash, 2021). Thus, any kind of behavioural alterations in orientation and locomotion, as observed in the present study, can be related to the impairment of sensory organ systems particularly the mechano and chemo-receptor systems. Sensory organs like lateral line, olfactory organs and membranous labyrinth helps the fishes in maintaining harmony with their environments and also control their vital behaviours (Mishra *et al*., 2011; Tiwari and Prakash, 2021). Hence, any impairment of these organs would produce behavioural changes in the fishes. Therefore, the ethological responses observed in dimethoate pesticide exposed fish, *Channa punctatus* might be contributing to the mortality in these stressed fishes.

Conclusion

The snake headed fish *Channa punctatus* is highly sensitive to the dimethoate, an organophosphate pesticide, as evidenced by behavioral responses like erratic swimming, a decrease in opercular activity, and increased surfacing and gulping activity to meet out the respiratory distress. In contrast to other air-breathing teleosts, the fish dies at low pesticide concentrations. Thus, it can be concluded that the primary reason of the decline of *Channa punctatus* in aquatic ecosystems is the over use of pesticides. In order to prevent contamination of our natural ecosystems, it is necessary to monitor the entry of pesticides from both point and nonpoint sources of pollution into our natural waterbodies. Biopesticides and natural products should be used in place of these agrochemicals.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, Sant Tulsidas P.G. College, Kadipur, Sultanpur, Uttar Pradesh, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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